

## إطالة فترة صلاحية منتجات الألبان باستخدام ثاني أكسيد الكربون المذاب

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تنحصر فترة صلاحية منتجات الألبان المبردة بفترة اسبوع إلى ثلاث اسابيع فقط . وهناك عدد من العوامل التي تساهم في جعل فترة الصلاحية هذه محدودة وتشمل الجودة الميكروبية للحليب الخام ،الانزيمات البكتيرية، ظروف المعاملات الحرارية، ودرجة حرارة المنتج اثناء عمليات التوزيع والنقل والخزن. ويمكن إستخدام ثاني اكسيد الكربون ( $CO_2$ ) للتأثير على هذه العوامل ولتحسين جودة منتجات الألبان المتنوعة ، حيث يعمل  $CO_2$  على تثبيط نمو وأيض مدى واسع من البكتيريا وخاصة تلك التي تتواجد في بيئة معامل الألبان.

هذه الورقة العلمية تبحث في أحدث التطورات في استخدام  $CO_2$  لتحسين جودة وإطالة فترة صلاحية منتجات الألبان وخاصة منتج الألبان المتخمر والمعروف بالزبادي ، كما تبحث في ميكانيكيات تثبيط  $CO_2$  للكائنات الدقيقة .وتقدم هذه الورقة العلمية عرضا لتأثير  $CO_2$  على الكائنات الدقيقة وعلى البكتيريا المكونه للأبواغ خلال المعاملات الحرارية وعلى انتاج ونشاط الانزيمات في الحليب بالإضافة إلى ذلك فان هذه الورقة العلمية تقدم عرضا لتأثير  $CO_2$  على جودة وصلاحية الزبادي والجوانب التكنولوجية لأستخدام  $CO_2$  في صناعة الزبادي.

# **Extending the Shelf-Life of Dairy Products by using Dissolved Carbon Dioxide**

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**The shelf-life of refrigerated dairy products is limited to 1 to 3 weeks. A number of factors contribute to this limited shelf-life: microbial quality of the raw milk, bacterial enzymes, thermal processing conditions, and distribution/storage temperatures. Carbon dioxide (CO<sub>2</sub>) can be used to influence these factors and improve the quality of a variety of dairy products. Growth and metabolism of a wide range of bacteria, particularly those found in the dairy processing environment, are inhibited in the presence of added CO<sub>2</sub>. This review presents the recent advances in the use of CO<sub>2</sub> for quality improving and shelf life extending of dairy product with special reference to the fermented dairy product the yogurt. Mechanisms of microorganism inhibition by CO<sub>2</sub> were discussed. Effects of CO<sub>2</sub> on microorganisms, spore-forming bacteria during thermal processing and enzyme production and activity in milk were presented. Furthermore, effects of CO<sub>2</sub> on yogurt quality and shelf- life and the technological aspects of CO<sub>2</sub> addition to yogurt were presented.**

## **Introduction**

The shelf life of nonsterile dairy products such as yogurt is generally limited to 1 to 3 weeks (Salvador and Fiszman 2004). This is could be depended upon the quality of the raw ingredients (Muir, 1996), bacterial enzymes (Champagne *et al.*, 1994) processing conditions (Lewis, 1999), and postprocessing handling (Henyon, 1999). Spoilage results primarily from the growth of organisms surviving pasteurization and/or postprocessing microbial contamination and degradative (proteolytic, lipolytic) enzymes surviving the thermal processing of the raw milk.

Several investigators have demonstrated that adding CO<sub>2</sub> to the atmosphere surrounding a product reduces the rate of growth of many food spoilage and pathogenic microorganisms (Hanlin and others 1995; Devlieghere and others 1998; Devlieghere and Debevere 2000) particularly those found in the dairy processing environment (Ruas-Madiedo *et al.*, 1996),. The largest inhibition occurs with gram-negative psychrotrophs, particularly *Pseudomonas* spp., and the least inhibition effect generally observed with gram-positive psychrotrophs, particularly *Lactobacillus* spp. (Hendricks and Hotchkiss 1997).

Here we present a review of scientific researches on using CO<sub>2</sub> in quality improvement and shelf life extending of the fermented dairy products with special reference to yogurt.

## **Mechanisms of microorganism inhibition by CO<sub>2</sub>**

There are at least four general mechanisms by which CO<sub>2</sub> inhibits microorganisms by affecting their growth and metabolism which includes:

1. Solubility of CO<sub>2</sub> in lipids may adversely affect membrane stability (Nilsson *et al.*, 2000; Ballestra *et al.*, 1996).
2. Hydration reactions of CO<sub>2</sub> result in reduced pH creating intracellular and environmental stress (Wolfe, 1980).
3. As a metabolite in many biochemical pathways, CO<sub>2</sub> can cause futile expenditure of cell energy (Dixon *et al.*, 1987).
4. CO<sub>2</sub> can cause physiochemical alteration and regulation of enzymes (King and Nagel, 1975; Pichard *et al.*, 1984).

Depending upon the growth medium, the organism, and its physiological state, a combination of these mechanisms is probably responsible for the observed effects.

## **Effects of CO<sub>2</sub> on microorganisms**

When CO<sub>2</sub> is dissolved in an aqueous medium it can retard the growth of Gram positive and Gram-negative organisms. The magnitude of the effect on the different phases of growth depends upon the organism (Table 1). For example, the lag phase of growth for *Pseudomonas fluorescens* increases with increasing concentrations of CO<sub>2</sub> (Hendricks and Hotchkiss, 1997). Other organisms are similarly affected but to different degrees: *Listeria monocytogenes* (Hendricks and Hotchkiss, 1997; Fernandez *et al.*, 1997), *Escherichia coli* (Martin *et al.*, 2003), *Bacillus licheniformis* (Martin *et al.*, 2003), and milk-borne psychrotrophs (Roberts and Torrey, 1988). Factors such as species, substrate, and CO<sub>2</sub> concentration influence the effect on pathogenic psychrotrophs (Bennik and others 1995).

## **Effects of dissolved CO<sub>2</sub> on spore-forming bacteria during thermal processing:**

Bacteria and their spores are more sensitive to thermal treatments under acidic conditions (Jay, 1992). When CO<sub>2</sub> is dissolved in an aqueous solution, such as milk, the pH decreases. The effects of CO<sub>2</sub> on the thermal resistance of vegetative cells and spores have been studied. The majority of work has been conducted in media at high pressures or near-supercritical conditions and at moderate to ambient temperatures.

The D<sub>89°C</sub>-value for *Bacillus cereus* spores was significantly decreased from 5.56 min in control milks (no added CO<sub>2</sub>) to 5.24 min in milk containing 33 mM CO<sub>2</sub> (Loss and Hotchkiss, 2002). A higher concentration of dissolved CO<sub>2</sub> (37 mM) in milk containing an initial inoculum of 8.7 log cfu/ml also resulted in fewer survivors (4.25 log cfu/ml) after a 15-minute treatment at 89°C compared to controls

that had 4.73 log cfu/ml survivors (Loss, 2001). After a 40-second treatment at 105°C, CO<sub>2</sub>-treated TSB (pH reduced to 6.3) had 1 log fewer survivors of *B. cereus* spores compared to untreated (pH 7.2) media heated for the same amount of time (Loss and Hotchkiss, 2003).

The effect of CO<sub>2</sub> on spore germination at higher heat treatments for shorter durations depends upon species and strain (Guirguis *et al.*, 1984). For example, 100% of spores of *B. subtilis* in reconstituted milk with pH adjusted to 5.86 with CO<sub>2</sub>, heated at 120°C for 2 s, survived compared to 0.1% survival of spores suspended in control milk that had no CO<sub>2</sub>. On the other hand, 2% of *B. cereus* spores heated at 125°C for 2 s in the CO<sub>2</sub>-treated milk survived compared to 100% survival in control milk.

Carbon dioxide (11.9 mM) dissolved in sterile milk packaged in glass jars and stored at 6°C for 35 days had no effect on the germination and outgrowth of *B. cereus* spores (Werner and Hotchkiss, 2002). It is suggested that moderate concentrations of CO<sub>2</sub> will not increase the risk of *B. cereus* spores growing in milk stored for extended periods of time. Concerns with the possibility of *Clostridium botulinum* germination, outgrowth and toxin production in CO<sub>2</sub>-treated milk prompted researchers to measure toxin production in milk inoculated with *C. botulinum* spores (Glass *et al.*, 1999). After a heat shock treatment, a cocktail of proteolytic and nonproteolytic strains of *C. botulinum* spores was inoculated into pasteurised milk containing 9.1 and 18.2 mM CO<sub>2</sub> or no added CO<sub>2</sub> (control) and stored for 6 days at abusive temperatures (21°C) and for 60 days at 6°C. Controls and CO<sub>2</sub>-treated milks stored at 21°C were grossly spoiled (SPC reaching 107 cfu/ml) at day 2 before *botulinum* toxin was detectable. Milk stored at 6°C, regardless of treatment, did not contain toxin over the 60-day storage period, leading to the conclusion that dissolved CO<sub>2</sub> as high as 18.2 mM in milk does not increase the risk of botulism.

### **Effects of CO<sub>2</sub> on enzyme production and activity in milk**

The effects of CO<sub>2</sub> on extracellular enzyme production by *P. fluorescens* in a simulated milk medium have been reported (Rowe, 1988). Carbon dioxide dissolved at 30 mM resulted in a 50% reduction in protease production at 7°C. After 5 days, lipase production was 85% greater in controls than in CO<sub>2</sub>-treated milk. This may have something to do with the increased solubility of CO<sub>2</sub> in lipids. More recently Habulin and Knez demonstrated that supercritical CO<sub>2</sub> (100 bar) can also significantly decrease the activity of a *P. fluorescens* lipase by 50% (Habulin and Knez, 2001).

### **Effects of CO<sub>2</sub> on Yogurt quality and shelf life**

Carbon dioxide is 'Generally Recognized As Safe' (GRAS; FDA, 2000). It can be used to extend the shelf-life of a variety of dairy products including fermented products such as yogurt.

Mold, yeast, spoilage bacterial growth and development of off-flavors can be a major determinant of shelf life of yogurts (Robinson and others 2002; Viljoen and others 2003). When using CO<sub>2</sub> to improve the quality and shelf life of fermented dairy products, the growth of beneficial bacteria such as lactic acid producers or probiotics must not be inhibited and at the same time spoilage organisms must be inhibited

in order to extend shelf-life. Two approaches have been taken to improve the quality of yogurts and cheeses:

1. Incorporate CO<sub>2</sub> into the raw milk to provide starting ingredients with good microbial quality.
2. Incorporate CO<sub>2</sub> into the final product or atmosphere surrounding the product to inhibit spoilage.

Important to both of these approaches is a low CO<sub>2</sub>/O<sub>2</sub> permeability of the package barrier.

To evaluate these strategies, researchers have monitored the growth and metabolism of fermentative bacteria in CO<sub>2</sub>-treated products and evaluated their quality compared to controls made by conventional methods. Yogurt made from CO<sub>2</sub>-treated raw milk (to a pH of either 6.0, 6.2, or 6.4) had similar sensory properties and viscosity as control yogurt but lower pH values after 7 days of storage at 7°C (Calvo *et al.*, 1999). In a different study growth and metabolism of two combinations of yogurt starter cultures in carbonated milk were monitored over a 49-day storage period at 4°C (Vinderola *et al.*, 2000). Carbon dioxide was added to the milk after the heat treatment of the raw milk and prior to inoculation with either of two starter culture blends:

1. *Lactobacillus acidophilus* and *Streptococcus thermophilus*
2. *L. acidophilus*, *S. thermophilus* and *Bifidobacteria bifidum*.

The growth of the first culture mixture was unaltered by the addition of CO<sub>2</sub> which reduced the pH from 6.84 to 6.31. In the presence of *B. bifidum* and CO<sub>2</sub> the counts of *L. acidophilus* were lower towards the latter part of the storage period. Concentrations of organic acids (pyruvic, lactic, and acetic) were the same in both CO<sub>2</sub>-treated and control milks for both culture combinations at the end of the storage period. However, acetic acid was lower in the CO<sub>2</sub>-treated milk containing *B. bifidum* during the first 4 weeks of storage, which may have been related to the lower counts of *L. acidophilus* in these samples later on. After 24 days the sensory properties of the yogurts, including mouthfeel, odour, acidity, and overall acceptability, were slightly improved in the CO<sub>2</sub>-treated yogurts. Carbon dioxide-treated milks reached the break point pH of 5.0 sooner than untreated milk (Vinderola *et al.*, 2000). Gueimonde *et al.* (2003) found that CO<sub>2</sub> dissolved in milk did not have a negative effect on the growth of probiotic bacteria. When CO<sub>2</sub> was dissolved directly into finished Swiss-style yogurt, the growth and viability of inoculated pathogens (*L. monocytogenes*, *E. coli*) and typical starter cultures were unaltered (Karagul-Yuceer *et al.*, 2001). In this study the CO<sub>2</sub> content was not measured directly so the actual amount dissolved is unclear. A consumer acceptance test demonstrated that the shelf-life of a yogurt beverage could be extended to 4 months with the addition of CO<sub>2</sub> (5 kg/cm<sup>2</sup> at 4°C) compared to uncarbonated controls that were spoiled at 30 days (Kosikowski and Choi, 1985). The yogurt beverages (fermented with *L. bulgaricus* and *S. thermophilus*) were packaged in glass containers and stored at 4.4°C and 10°C. After 40 days yeast and mould counts increased from 10 cfu/g to 100 and 200 cfu/g in uncarbonated yogurt beverages stored at 4.4 and 10°C respectively, whereas in the carbonated product they remained below 10 cfu/g over an 80-day period at both storage temperatures. The soluble protein and volatile fatty acid content of the control yogurts increased at a faster rate than the carbonated samples, an indication that spoilage was occurring

more rapidly, though unfortunately SPC were not measured. The noncarbonated yogurt pH dropped faster than the carbonated yogurt pH, indicating slowed metabolism of the lactic acid bacteria (LAB), but their growth was not measured in the different treatments.

Karagul-Yuceer and others (2001) recently reported that high levels (1.1 to 1.2 volumes) of dissolved CO<sub>2</sub> incorporated into yogurt had little effect on desirable typical or nontypical starter cultures microorganisms. It had been hypothesized that the addition of CO<sub>2</sub> to the product could feasibly stimulate growth of starter bacteria, reducing production time. Recently, Ansari et al., (2013) studied the effects of CO<sub>2</sub> addition to raw milk on microbial, physiochemical and sensory properties of probiotic set yoghurt. They concluded that, the CO<sub>2</sub>-treatment of milk had no significant effect on pH of yoghurts. CO<sub>2</sub>-treatment improved technological properties of yoghurts: syneresis was reduced and viscosity was increased. Also CO<sub>2</sub>-treatment slightly improved growth and viability of the starter microorganisms during cold storage.

### **Technological aspects of yogurt CO<sub>2</sub> addition**

A method whereby spoonable yogurt could be carbonated has been patented and modifications of a more optimized method and model for carbonation of viscous fluids have been published (Taylor and Ogden 2002). The economic investment in equipment and supplies is minimal, with packaging costs the most significant expense. Headspace flushing of yogurt packages with CO<sub>2</sub> can extend shelf life by inhibiting spoilage organisms (Loss and Hotchkiss, 2003), and it is possible that direct incorporation of CO<sub>2</sub> into the product may also beneficially impact shelf life.

The direct injection of 5.68 to 22.7 mM CO<sub>2</sub> into products coupled with high barrier packaging has been developed as a method to inhibit undesirable microorganisms in dairy products and thus extend shelf life (Chen and Hotchkiss 1991). Liquefied or compressed CO<sub>2</sub> gas can be incorporated directly into a flowing stream of product via a gas-sparging unit, a process commercially practiced in several areas of the world. The device that is most often employed consists of a sintered stainless steel frit with porosity in the range of 7 to 30  $\mu$ m. The process has been termed directly sparged in-line "direct addition of carbon dioxide (DAC)" in order to distinguish it from conventional modified atmosphere packaging (MAP). The gas is added to the product for the purpose of increasing shelf life by inhibiting microbial activity. The cost of the addition of CO<sub>2</sub> to dairy foods via this method is generally economically feasible, and the incorporation of CO<sub>2</sub> typically occurs within the normal stream of product in a production system. Only a minimal one-time investment is required for equipment, and the cost of CO<sub>2</sub> gas is low; the most significant and recurring cost involved is in barrier packaging.

Several authors have pointed out that in extending shelf life, atmospheric CO<sub>2</sub> first dissolves in the undissociated form into the liquid phase of the product before inhibiting respiratory and microbial systems (Loss and Hotchkiss, 2003, Hotchkiss et al., 2006). Thus, CO<sub>2</sub> in the atmosphere if incorporated with MAP is not the effective agent *per se* in the inhibition of microorganisms. The CO<sub>2</sub> must first

dissolve into the product and eventually into microbial cells. The amount of CO<sub>2</sub> dissolved in water is governed by the partial pressure of the CO<sub>2</sub> above the water as well as the amount of CO<sub>2</sub> available, which is determined by both the volume of the headspace and the concentration of CO<sub>2</sub> in that headspace (Hotchkiss et al., 2006).

Rather than rely on an equilibrium being established between the headspace in a package and the product, it has been suggested that the direct addition of CO<sub>2</sub> into products may result in improved microbial control by ensuring a constant low concentration of dissolved CO<sub>2</sub> (Gorski 1996). Henry's law illustrates that as the aqueous concentration of CO<sub>2</sub> increases, the partial pressure of CO<sub>2</sub> ( $p\text{CO}_2$ ) increases accordingly at a fixed temperature. If the temperature of the product is controlled, the concentration of CO<sub>2</sub> within the aqueous liquid will remain constant, assuming a closed system and no loss of CO<sub>2</sub>. This process has advantages over conventional MAP in that no headspace is required and the amount of dissolved CO<sub>2</sub> can be carefully controlled.

## **Conclusion**

The relatively short shelf life and rapid loss of quality has necessitated the requirement of an increased shelf life for many dairy products. Carbon dioxide is a unique natural antimicrobial and processing aid that has several potential uses in the dairy industry. It is unique because it can be added to and removed from dairy products with no deleterious effects. It is GRAS, and at the present time does not need to be declared on an ingredient label.

The direct addition of CO<sub>2</sub> to dairy products coupled with increasing the barrier properties of the containers has been commercially successful and economically feasible with some dairy products. Substantial research exists to show that direct addition of CO<sub>2</sub> to milk prior to processing or further manufacturing to yogurt can significantly improve and extend the shelf life of these products, increase product safety, and in some cases improve product quality. The cost of the addition of CO<sub>2</sub> to dairy foods via DAC method is generally economically feasible, and can be incorporated within the normal stream of product in a production system.

**Table 1: Effects of CO<sub>2</sub> on bacterial growth (measured by conductance)  
described with the Gompertz model (adapted from Martin *et al.*, 2003)**

Organism(s)	[CO <sub>2</sub> ] (mM)	<i>R</i> <sub>2</sub>	Growth rate (°S/h)	Time to max. growth rate (h)	Max. change in conductance (°S)	Doubling time (h)	Lag time (h)
Raw milk microflora	0.6	1.0	0.200 <sub>a</sub>	26.0 <sub>a</sub>	88.9 <sub>a</sub>	1.8	20.0
	15.4	1.0	0.132 <sub>b</sub>	33.0 <sub>b</sub>	92.2 <sub>b</sub>	2.3	25.4
	27.9	1.0	0.135 <sub>c</sub>	40.2 <sub>c</sub>	98.0 <sub>c</sub>	2.2	32.8
	38.6	0.99	0.133 <sub>d</sub>	44.3 <sub>d</sub>	80.5 <sub>d</sub>	2.3	37.7
<i>P. fluorescens</i>	44.5	1.0	0.113 <sub>e</sub>	52.9 <sub>e</sub>	87.9 <sub>e</sub>	2.7	44.1
	0.4	0.99	0.112 <sub>a</sub>	11.7 <sub>a</sub>	78.2 <sub>a</sub>	2.7	3.3
	11.2	0.99	0.128 <sub>b</sub>	21.1 <sub>b</sub>	69.2 <sub>b</sub>	2.4	13.3
	27.1	0.99	0.130 <sub>b</sub>	22.7 <sub>c</sub>	59.9 <sub>c</sub>	2.3	15.0
	33.6	0.99	0.088 <sub>c</sub>	27.3 <sub>d</sub>	61.9 <sub>d</sub>	3.4	16.0
	46.3	0.99	0.088 <sub>c</sub>	37.5 <sub>e</sub>	65.6 <sub>e</sub>	3.4	26.1
	0.5	0.99	0.064 <sub>a</sub>	47.6 <sub>a</sub>	56.0 <sub>a</sub>	4.7	29.4
<i>E. coli</i>	49.4	0.97	0.055 <sub>b</sub>	53.8 <sub>b</sub>	22.0 <sub>b</sub>	5.5	38.1
<i>L. monocytogenes</i>	0.5	0.98	0.136 <sub>a</sub>	22.6 <sub>a</sub>	118.0 <sub>a</sub>	2.2	15.2
	48.9	0.99	0.100 <sub>b</sub>	44.4 <sub>b</sub>	71.5 <sub>b</sub>	3.0	34.4
<i>Enterococcus faecalis</i>	0.5	0.99	0.055 <sub>a</sub>	51.8 <sub>a</sub>	69.0 <sub>a</sub>	5.5	33.6
	51	0.98	0.076 <sub>b</sub>	50.7 <sub>b</sub>	40.7 <sub>b</sub>	4.0	37.6
<i>B. cereus</i>	0.5	1.00	0.128 <sub>a</sub>	33.9 <sub>a</sub>	79.3 <sub>a</sub>	2.4	26.1
	47.1	0.99	0.105 <sub>b</sub>	37.6 <sub>b</sub>	77.9 <sub>b</sub>	2.9	28.1
	61.4	0.99	0.057 <sub>c</sub>	44.4 <sub>c</sub>	74.8 <sub>c</sub>	5.3	26.7
<i>B. licheniformis</i>	0.5	0.99	0.057 <sub>a</sub>	48.4 <sub>a</sub>	51.4 <sub>a</sub>	5.3	30.9
	49.4	0.96	0.057 <sub>a</sub>	54.1 <sub>b</sub>	31.2 <sub>b</sub>	5.2	36.7

<sup>a-e</sup> For each organism, different superscript letters denote that parameters are statistically different from each other ( $\alpha=0.05$ )



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